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TRYPTAMINES
I. THE CHROMATOGRAPHY OF MELATONIN

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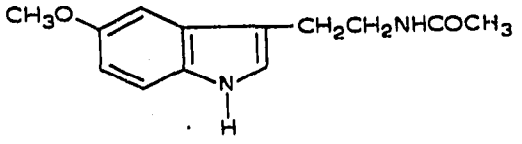
SUMMARY

An investigation of the gas–liquid chromatography of melatonin and other tryptamines has shown that temperature programming of the parent compounds and of their silyl derivatives may be used to advantage. Lower column temperatures may be used with the silyl and trifluoroacetyl derivatives.

INTRODUCTION

The indole derivative, melatonin (N-acetyl-5-methoxy-tryptamine) has been characterized as the active constituent (hormone) of the pineal gland[1]. The hormone is responsible for the lightening of skin colour of tadpoles and toads following perfusion with extracts of the gland. Considerable interest has been attached to melatonin in view of the extremely small amounts required to exert this effect[2–4].



Melatonin

Since the material is present in tissues in microquantities only, separation procedures rest on chromatographic techniques. The present study has been concerned with the recovery of melatonin from biological fluids and aspects of the gas–liquid chromatography of the material are now reported.

Previous records of the behaviour of melatonin during gas chromatography clearly indicate that the technique must contend with the polarity and relative involatility of the compound, leading to use of high temperatures (above 200°) still accompanied by relatively long retention times[5,6].

A study has now been made of the temperature–time relationships for melatonin, together with its silyl and trifluoroacetyl derivatives. From information gained in isothermal procedures temperature-programmed conditions have been developed. Accordingly, starting with column temperatures about 160–180° and isothermal

periods of 6–10 min, the effect has been examined of applying different rates of heating, 10–20°/min, up to 240°.

EXPERIMENTAL PROCEDURE

Materials

Melatonin and other indoles were chromatographically pure.

Trimethylsilyl derivatives were prepared by treating the indoles (0.5 mg) in pyridine solution (0.2 ml) with N,O-*bis*-(trimethylsilyl)acetamide (BSA 0.1 ml) for 1 min at 80°. In a similar manner trifluoroacetyl derivatives were prepared in pyridine solution by treating with trifluoroacetic anhydride for 1 min at 50°. Reaction mixtures were used directly for the chromatography.

Procedure

Columns (glass 120 × 0.3 cm I.D.) consisted of 3% SE-30 (methylsilicone) or 3% DC-550 (methylsilicone with 25% phenylsilicone) applied by the filtration technique to 100–120 mesh Celite, previously acid washed and treated with hexamethyldisilazane. Chromatography at the various temperatures was carried out using nitrogen at a flow rate of 60 ml/min as carrier gas and a flame ionization detector.

RESULTS

Retention times for melatonin and its trimethylsilyl and trifluoroacetyl derivatives are shown in Table I for the SE-30 and DC-550 columns. Comparisons of the

TABLE I

INFLUENCE OF COLUMN TEMPERATURE ON THE RETENTION TIMES[a] ON SILICONE COLUMNS OF MELATONIN AND ITS SILYL AND TRIFLUOROACETYL DERIVATIVES

Column temperature	*Melatonin*		*Silyl derivative*		*Trifluoroacetyl derivative*	
	SE-30 column	*DC-550 column*	*SE-30 column*	*DC-550 column*	*SE-30 column*	*DC-550 column*
180	–	–	26.4	–	5.0	–
190	–	–	16.8	35.0	–	–
200	12.8	16.2	11.0	15.0	2.0	
210	8.6	10.4	7.0	7.0		
220	5.9	6.3	5.0	3.8		
230	4.4	4.3	3.4	2.5		
240	3.1	3.0	2.5	1.8		
250	2.3	2.0	1.7	1.2		
260	1.5	1.2				

[a] Expressed in minutes.

retention times on the SE-30 column of melatonin and other tryptamines, together with their trimethylsilyl and trifluoroacetyl derivatives are shown in Table II.

Conditions and results of temperature-programming experiments are shown in Tables III and IV, respectively, while curves illustrative of isothermal and programmed separations are shown in Fig. 1.

TABLE II

INFLUENCE OF COLUMN TEMPERATURE ON THE RETENTION TIMES[a] OF INDOLES AND THEIR DERIVATIVES, SE-30 COLUMN

I = Tryptamine; II = 5-methoxy tryptamine; III = N,N-dimethyl tryptamine; IV = bufotenin; V = seratonin; VI = melatonin.

Column temperature (°C)	*Indoles*						*Silyl derivatives*						*Trifluoroacetyl derivatives*					
	I	*II*	*III*	*IV*	*V*	*VI*	*I*	*II*	*III*	*IV*	*V*	*VI*	*I*	*II*	*III*	*IV*	*V*	*VI*
180													0.9	1.1		2.4	1.7	5.0
													1.6	1.5			2.1	
													3.0	2.8			4.2	
200	1.55	3.2	1.9	4.7		12.0	3.0	4.0	1.3	3.4	10.6	7.8	0.5	0.8		1.2	0.6	2.0
													0.8	1.1			0.9	
													1.2	1.4			1.6	
220	1.1	1.8	1.2	2.4		5.0	1.6	2.0	0.7	1.7	4.8	3.6						
240	0.65	1.0	0.7	1.25		2.7	0.8	1.0	0.4	0.9	2.2	1.9						
260	0.45	0.65	0.5	0.8		1.5												

[a] Expressed in minutes.

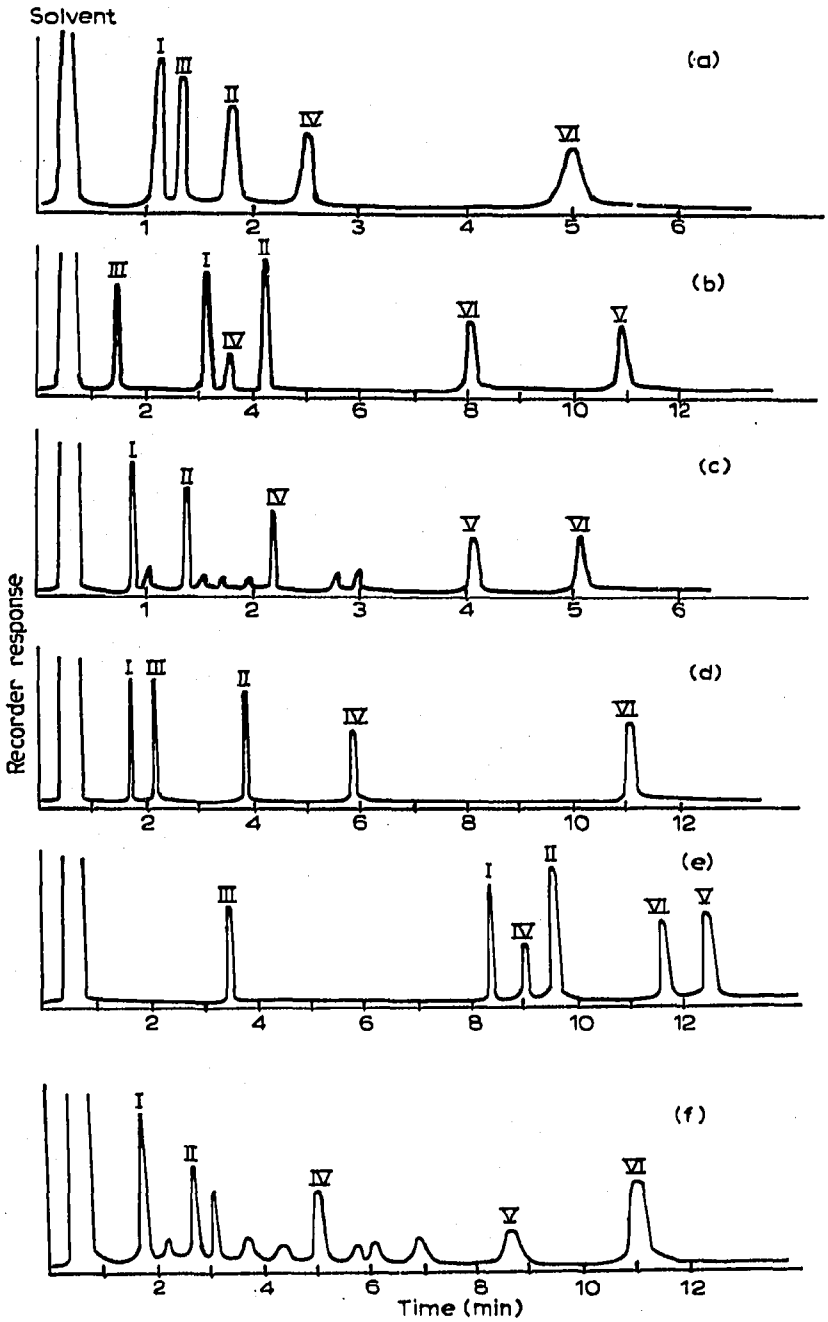


Fig. 1. Gas–liquid chromatograms of indoles. (a) Isothermal separation of indoles at 220°. (b) Isothermal separation of silyl derivatives at 200°. (c) Isothermal separation of trifluoroacetyl derivatives at 180°. (d) Temperature-programmed separation of indoles. Initial temperature, 180°; isothermal period, 6 min; rate of heating, 15°/min; final temperature, 240°. (e) Temperature-programmed separation of silyl derivatives. Initial temperature, 160°; isothermal period, 4 min; rate of heating, 10°/min; final temperature, 240°. (f) Temperature-programmed separation of trifluoroacetyl derivatives. Initial temperature, 160°; isothermal period, 8 min; rate of heating, 10°/min; final temperature, 190°. Roman figures are explained in Table II.

TABLE III

TEMPERATURE PROGRAMMING CONDITIONS

	Run No.									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Initial temperature (°C)	180	180	180	180	190	190	190	190	190	200
Period of isothermal run (min)	6	10	8	10	2	3	4	5	3	3
Rate of heating (C°/min)	15	20	10	15	10	10	10	10	15	10
Final temperature (°C)	240	240	240	240	240	240	240	240	240	240

DISCUSSION

Previous investigations on the chromatography of melatonin have employed SE-30 columns but with retention times of the order of 30 min (ref. 6). High column temperature (265°) has been used to overcome this disadvantage and reduce the retention time to 3 min (ref. 7). The use of mixed stationary phases (silicone F60 (7%) with ethylene glycol succinate polymer (1%)) at 216° also gave long retention times[5].

Present results favour the use of non-polar columns. As shown in Table I even the slightly modified DC-550 stationary phase at temperatures up to 220° gives increased retentions for melatonin. However, owing to differences in the rate of change in retention times on the SE-30 and DC-550 columns with further increase in temperature (above 220°), retention times on the two columns become practically the same or may even favour the latter phase.

TABLE IV

TEMPERATURE PROGRAMMED SEPARATION OF MELATONIN FROM OTHER INDOLES ON A 3% SE-30 COLUMN

Compound	*Retention times (min) of run No.*									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Tryptamine	1.7	1.9	1.9	1.9	1.3	1.3	1.3	1.3	1.3	1.0
N,N-Dimethyl tryptamine	2.0	2.2	2.2	2.2	1.6	1.6	1.6	1.6	1.6	1.6
5-Methoxy tryptamine	3.8	4.1	4.3	4.3	2.8	2.9	3.0	2.9	2.9	2.1
Bufotenin	5.6	6.0	6.7	6.3	3.8	4.1	4.2	4.2	4.1	2.9
Melatonin	10.6	13.3	13.1	13.9	6.6	7.7	8.6	9.4	7.4	6.2

Below the optimum temperature melatonin gives broadened peaks unsatisfactory for quantitative work. Thus at 250° the peak width at half height was 1.5 mm, broadening to 12.0 mm at 200° for the same quantity. The limit of detection was 0.01 μg.

Polar stationary phases, such as diethylene glycol succinate, were also unsatisfactory, giving long retention times and much tailing, while mixed phases led to difficulty in reproducing retention values.

Table II shows that melatonin is easily distinguished from the other indoles by its larger retention time and suggests that programming could be used to advantage.

Indeed Figs. 1a and b show that even better separation of melatonin from other indoles results from this technique.

In common with other indoles melatonin resists silylation by mild reagents such as hexamethyldisilazane. With the more active reagent, BSA, silylation was achieved at 80°, probably at the ring nitrogen. Formation of the silyl derivatives led to shorter retention times, even at lower temperatures, with the more marked rate of change again apparent for the DC-550 column. Sharp peaks were obtained down to 170° for these derivatives. At any given temperature a better peak shape was obtained for silylated melatonin than for the parent compound. In addition, a further improvement in separation of melatonin was achieved by programming for the silyl derivative (Figs. 1c and d).

The position with the trifluoroacetyl derivatives is not yet satisfactory. It is clear from Table II that even lower temperatures may be employed with these derivatives. Thus the promise of shorter retention times indicated at 180° was clearly confirmed for the series of derivatives at 200°. However, the position is clouded by the fact that several materials gave three peaks, a result that will require a separate investigation for complete resolution of the products.

APPLICATION TO BIOLOGICAL MATERIALS

The stability of melatonin lends itself to use of continuous extraction techniques for separation from biological fluids. Following addition of melatonin over the range 1–20 μg/ml to serum, recoveries of the order of 85–90%, as judged by peak heights, were obtained using ethyl acetate as solvent for liquid–liquid extraction from the diluted serum (1 + 9) over a period of 2 h.

The residue obtained by removal of the solvent in a rotary evaporator was redissolved in ethyl alcohol (0.5 ml) for introduction (1–2 μl according to concentration) into the chromatograph.

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